PB84-102409

Dose Determinations for Waterborne 2,5,2',5'-(14C)Tetrachlorobiphenyl and Related Pharmacokinetics in Two Species of Trout 'Salmo gairdneri and Salvelinus fontinalis' A Mass-Balance Approach

(U.S.) Environmental Research Lab.-Duluth, MN

1983

U.S. Department of Commerce
National Technical Information Service
NTIS

TECHNICAL REPORT DATA

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1. REPORT NO.	2.	3.
EPA-600/J-83-066		PB84-102409

4. TITLE AND SUBTITLE
Dose Determinations for Waterborne 2,5,2',5'-[^{14}C] Tetrachlorobiphenyl and Related Pharmacokinetics in Two Species of Trout (Salmo gairdneri and Salvelinus fontinalis): A Mass-Balance Approach

5. REPORT DATE
1983

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
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8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
U.S. Environmental Protection Agency
Environmental Research Laboratory-Duluth
6201 Congdon Boulevard
Duluth, Minnesota 55804

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.

12. SPONSORING AGENCY NAME AND ADDRESS
Same as above

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA/600/03

15. SUPPLEMENTARY NOTES
Toxicol. Appl. Pharmacol. 68, 177-187 (1983)

16. ABSTRACT

A mass-balance study was undertaken to evaluate the accuracy of dose determinations of waterborne 2,5,2',5'-[^{14}C] tetrachlorobiphenyl (TCB) made on transected brook trout (Salvelinus fontinalis) and rainbow trout (Salmo gairdneri) and to determine any pharmacokinetic differences between the two species. The total calculated [^{14}C] TCB absorbed by brook (17.8 μg) and rainbow (24.5 μg) trout was compared to the actual body burden measurements of [^{14}C] TCB for brook (17.4 μg) and rainbow (25.6 μg) trout; the latter measurements also included excretory losses through the urine, feces, and across the gill surface. Approximately 1% of the total dose was excreted of which 75% was in the urine and 25% in the feces. The agreement between the whole body burden measurements of [^{14}C] TCB and the total calculated micrograms of [^{14}C] TCB absorbed was within 10% in both species. Mass-balance measurements were converted to dose by dividing by fish weight. Mean calculated and measured doses were 31.4 and 30.4 μg/kg/48 hr for brook trout and 32.3 and 33.6 μg/kg/48 hr for rainbow trout. No species differences were seen in either calculated or measured doses or in total radioactivity excreted. Respiratory function between the two species was similar except for a significantly higher mean ventilation rate for brook trout (84 ± 14/min) than for rainbow trout (65 ± 4/min). There were also no fluctuations noted in either respiratory function or [^{14}C] TCB uptake efficiency across the gills over the 48-hr exposure period. These studies demonstrated the ability to accurately calculate a water dose in micrograms per kilogram per hour for individual fish that could be directly compared to other fish species or to mammals.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	12
	20. SECURITY CLASS (This page)	22. PRICE
	UNCLASSIFIED	

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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EPA-600/J-83-066
JOURNAL ARTICLE

TOXICOLOGY AND APPLIED PHARMACOLOGY 68, 177–187 (1983)

Dose Determinations for Waterborne 2,5,2′,5′-[^{14}C]Tetrachlorobiphenyl and Related Pharmacokinetics in Two Species of Trout (*Salmo gairdneri* and *Salvelinus fontinalis*): A Mass-Balance Approach

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Received July 26, 1982; accepted December 20, 1982

Dose Determinations for Waterborne 2,5,2′,5′-[^{14}C]Tetrachlorobiphenyl and Related Pharmacokinetics in Two Species of Trout (*Salmo gairdneri* and *Salvelinus fontinalis*): A Mass-Balance Approach. MCKIM, J. M., AND HEATH, E. M. (1983). *Toxicol. Appl. Pharmacol.* **68,** 177–187. A mass-balance study was undertaken to evaluate the accuracy of dose determinations of waterborne 2,5,2′,5′-[^{14}C]tetrachlorobiphenyl (TCB) made on transected brook trout (*Salvelinus fontinalis*) and rainbow trout (*Salmo gairdneri*) and to determine any pharmacokinetic differences between the two species. The total calculated [^{14}C]TCB absorbed by brook (17.8 μg) and rainbow (24.5 μg) trout was compared to the actual body burden measurements of [^{14}C]TCB for brook (17.4 μg) and rainbow (25.6 μg) trout; the latter measurements also included excretory losses through the urine, feces, and across the gill surface. Approximately 1% of the total dose was excreted of which 75% was in the urine and 25% in the feces. The agreement between the whole body burden measurements of [^{14}C]TCB and the total calculated micrograms of [^{14}C]TCB absorbed was within 10% in both species. Mass-balance measurements were converted to dose by dividing by fish weight. Mean calculated and measured doses were 31.4 and 30.4 μg/kg/48 hr for brook trout and 32.3 and 33.6 μg/kg/48 hr for rainbow trout. No species differences were seen in either calculated or measured doses or in total radioactivity excreted. Respiratory function between the two species was similar except for a significantly higher mean ventilation rate for brook trout (84 ± 14/min) than for rainbow trout (65 ± 4/min). There were also no fluctuations noted in either respiratory function or [^{14}C]TCB uptake efficiency across the gills over the 48-hr exposure period. These studies demonstrated the ability to accurately calculate a water dose in micrograms per kilogram per hour for individual fish that could be directly compared to other fish species or to mammals.

In aquatic toxicology, one of the major routes of entry of xenobiotic chemicals dissolved in water is directly across the gills of fish. Since special transport systems are usually not active for trans-gill transport of man-made chemicals, the movement is mainly through passive diffusion (Hunn and Allen, 1974). The rate of this passive flux across the gills is controlled by chemical and electrical gradients and by the physical properties of the chemical (Hunn and Allen, 1974; Neely *et al.*, 1974; Yang and Sun, 1977; Veith *et al.*, 1979). The total flux of these waterborne chemicals is also affected by the volume of water pumped across the gills, which depends on metabolic rate (Murphy and Murphy, 1971; Jones and Randall, 1978; Boddington *et al.*, 1979; Rogers and Beamish, 1981; McKim and Goeden, 1982).

Until recently, it was not possible to determine the actual dose of a waterborne chemical received across the gills of a fish. This void made it difficult to compare toxicity data on fish receiving a waterborne exposure of a chemical expressed in micrograms per liter of water outside the animal with that of

0041-008X/83 $3.00
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mammals, since mammalian data are generally expressed as a dose given directly into the animal (micrograms per kilogram). It is also difficult to compare the sensitivity of several species of fish, when the actual amount of chemical absorbed from the water for each species is not known. Estimates of a waterborne dose can be made by measuring the total body concentration of a chemical at specific points in time; but with readily metabolized chemicals these estimates would be in error unless a mass-balance determination, including water uptake and excretion, was completed on each chemical. An apparatus for directly measuring the dose of a waterborne chemical in fish was developed by McKim (1981) and McKim and Goeden (1982). Their respirometer–metabolism chamber allowed a direct measure of the inward flux of a chlorinated hydrocarbon (endrin) from inspired water during a single pass across the gills and allowed the determination of an endrin dose (micrograms per kilogram per day). These early experiments, however, did not include a mass-balance accounting of the endrin absorbed across the gills and were based entirely on the amount removed from the respiratory water.

In the present comparative study, a complete mass balance of 2,5,2′,5′-[^{14}C]tetrachlorobiphenyl (TCB) over a 48-hr exposure period was completed for both brook and rainbow trout and involved direct water uptake and excretion. This mass-balance approach was designed to further evaluate and validate the accuracy of gill mass transfer dose measurements and to provide a comparative respiratory function, pharmacokinetic study of [^{14}C]TCB in two species of trout. The high lipid solubility and relatively slow metabolism of TCB provided an ideal chemical for these mass-balance studies.

METHODS

Biological

Animals. The rainbow (*Salmo gairdneri*) and brook (*Salvelinus fontinalis*) trout used in this study weighed 660 to 840 and 530 to 640 g, respectively. They were maintained on Glencoe Mills[1] trout food for up to 24 hr before experimentation. The fish were kept on a 12-hr photoperiod (incandescent) at an intensity of 11.0 lm at the water surface. All of the trout were acclimated to a temperature of 11 to 12°C for several months prior to experimental use.

Metabolism chamber and surgical procedures. Fish were exposed to aqueous 2,5,2′,5′-[^{14}C]tetrachlorobiphenyl (TCB) in a Plexiglas respirometer–metabolism chamber described in detail by McKim and Goeden (1982). Briefly, an anesthetized fish (tricaine methanesulfonate (MS222) 100 mg/liter, Finquel[2]) was immobilized by spinal transection, and 48 hr later reanesthetized and placed on a surgical table (McKim and Syrett, 1982). Then a latex rubber oral membrane was sutured around the mouth to separate inspired and expired water. Next, a urinary catheter (polyethylene tubing, PE-60), followed by a fecal catheter, was sutured in place. The fecal catheter was constructed of a 10-cm-long Teflon tube (5 mm o.d.) with a beveled anterior end and a small latex rubber bag attached to the posterior end. The anterior end was inserted 3 cm into the anal opening and a purse string suture was used to hold it firmly in place. The fish was then removed from the surgical table and placed into the respirometer–metabolism chamber. Exposure to [^{14}C]TCB began 24 hr later.

Sample collection. During each 48-hr exposure period, eight or nine measurements were made on each fish for ventilation rate, heart rate, and ventilation volume (McKim and Goeden, 1982). In addition, dissolved oxygen (DO) concentrations in inspired and expired water were measured with a Beckman[3] oxygen electrode to determine oxygen utilization, i.e., percentage of oxygen removed from water passing across the gills. The [^{14}C]TCB concentrations from inspired and expired water were measured after hexane extraction (described under water analyses) followed by analysis to determine the percentage uptake efficiency of [^{14}C]TCB. Uptake efficiency is the percentage decrease in chemical concentration between the inspired and expired water. At the end of each 48-hr exposure, dosing was stopped, the metabolism chamber flushed out, and the expired water monitored for 1 hr, as above, for the excretion of TCBs across the gills.

To verify that an accurate measurement of uptake efficiency was made, the system was checked with an oral membrane alone and with the membrane attached to a dead fish. No differences in TCB concentration between incoming and outgoing water were detected in either case suggesting that no significant absorption other than gill absorption was contributing to the uptake efficiency measurements.

Urine was collected via the catheter into a polyethylene

[1] Glencoe Mills, Glencoe, Minn. 55336.
[2] Ayerst Laboratories, N.Y.
[3] Beckman Instruments Co., Skokie, Ill. 60076.

bottle; both volume and total radioactivity were measured at 24 and 48 hr. At the end of the 48-hr exposure period, each fish was killed. Blood was drawn by caudal puncture to measure hematocrit and total radioactivity in whole blood, plasma, and packed cells. Feces were collected by removing the gut with the fecal catheter attached and flushing the contents into a beaker with distilled water. Finally, bile was collected by gall bladder puncture, and volume was measured. All samples, as well as the carcass of each fish, were frozen at −20°C until analyzed for ^{14}C.

Physical Chemical

Water characteristics. The water, obtained directly from Lake Superior, was maintained at 11 ± 1°C. Water chemistry characteristics of the incoming Lake Superior water during the experimental period (November 1980 to January 1981) were pH, from 7.2 to 7.6 (n = 7); alkalinity, $\bar{X}$ ± SD 39.5 ± 1.4 mg/liter $CaCO_3$ (n = 7); hardness, $\bar{X}$ ± SD 42.9 ± 1.0 mg/liter $CaCO_3$ (n = 7); and dissolved oxygen, from 10.5 to 11.0 mg/liter (n = 56).

[^{14}C]TCB dosing apparatus. Ring-labeled 2,5,2',5'-[^{14}C]tetrachlorobiphenyl (Mallinckrodt[4]) in benzene, with a specific activity of 9.87 mCi/mmol, was used for these experiments. The [^{14}C]TCB benzene solution was checked for concentration and purity (>99%) by gas chromatography (GC) with a series of cold TCB standards. An automated GC system (Hewlett-Packard[5]) was fitted with a flame ionization detector and a 2 m × 2-mm-i.d. glass column packed with OV-17 and QF-1 (1.5 and 1.95%) on 80–100 mesh Gas Chrom Q. The GC conditions were detector, 300°C; injector, 250°C; and column, 220°C.

A stock solution of [^{14}C]TCB was introduced into the respirometer–metabolism chamber by a series of mixing cells (McKim and Goeden, 1982). Water at 11°C flowed into the toxicant mixing cell through a small float valve. A stock solution for each experiment was prepared by mixing [^{14}C]TCB in benzene with distilled water in a 20-liter glass carboy. This stock solution was delivered by an FMI[6] chemical metering pump at 2 ml/min to the toxicant mixing cell (McKim and Goeden, 1982) where it was diluted with the Lake Superior water at a flow rate of 600 ml/min. A new stock solution was prepared for each 2-day experiment. The [^{14}C]TCB and water were well mixed in the mixing cell. From this point, the [^{14}C]TCB solution flowed from the mixing cell through flow calibrated capillary tubes, that deliver 300 ml/min, to two respirometer–metabolism chambers operated simultaneously.

The [^{14}C]TCB concentrations in inspired and expired water were measured following hexane extraction. Two hundred and twenty-five milliliters of tank water and 25 ml of hexane were vigorously stirred for 30 min in a 250-ml volumetric flask. A 5-ml aliquot of the hexane layer was combined with a nonaqueous mixture (Instafluor[7]), and total radioactivity was counted. Spiked samples gave 100 ± 3% (n = 6) recovery by this method. The nominal [^{14}C]TCB concentration in inspired water was 0.10 μg TCB/liter, and the nominal benzene concentration was 0.02 μl/liter. The mean measured water concentrations of [^{14}C]TCB are given for each fish in Table 2. The TCB concentrations used in these experiments were approximately 33 times less than the chronic no effect concentration established by a fish life-cycle exposure (DeFoe *et al.*, 1978).

Tissue analysis. After the blood, bile, and feces were sampled, the whole fish was coarsely ground in a meat grinder and homogenized in a Waring blender. This homogenate was then used for GC and liquid scintillation counting (LSC). A 10-g sample of the whole fish homogenate was prepared for GC analysis by mixing with 170 Na_2SO_4 (anhydrous) and then extracted with 180 ml of redistilled hexane and methylene chloride (50:50) for 12 hr in a Soxhlet extraction apparatus. Each sample was concentrated to 20 ml in a hood, and three 2-ml aliquots were taken for lipid analysis. These extracts were dried to constant weight at 40°C in tared foil pans. Lipids were removed from the remaining extract in 20 g Florisil in a 20-mm-i.d. glass column; TCB was eluted with 50 ml of hexane three times. The extract was concentrated to 7 ml in a Kuderna-Danish evaporator; TCB was quantified by GC.

Each whole fish homogenate was also analyzed for total ^{14}C by weighing four subsamples of each fish (100 to 250 mg) in glass scintillation vials. The samples were digested with 2 ml of Soluene-350[7]: isopropanol (1:2) at 40°C for 24 to 72 hr, decolorized with 500 μl of a 30% hydrogen peroxide solution, and then counted after adding 15 ml acidified Instagel[7] (9 parts Instagel: 1 part 0.5 N HCl). A similar procedure was used to prepare whole blood, packed blood cells, and bile in sample volumes of 250, 100, and 25 μl, respectively.

Fecal samples were homogenized in a glass tissue homogenizer and diluted to 40 ml with distilled water. From this homogenate, four 2-ml samples were pipetted into tared scintillation vials and dried to constant weight in a vacuum oven at 40°C. No loss of radioactivity was found during the drying step. After weighing, the samples were rehydrated with 100 ml of distilled water for 30 min at 40°C, digested, bleached, and counted as described above. Plasma and urine samples were counted by adding 10 ml Instagel to 1 ml of sample.

All samples were counted at room temperature in a Beckman LC-8000[8] counter set at a 2% counting error (2σ). A computer program was used to calculate total ^{14}C

[4] Mallinckrodt, Inc., P.O. Box 5439, St. Louis, Mo. 63160.

[5] Hewlett-Packard, Avondale, Ill.

[6] Fluid Metering, Inc., Oyster Bay, N.Y. 11771.

[7] Packard Instruments, Chicago, Ill.

[8] Beckman Instruments, Skokie, Ill. 60076.

based on quench curves generated from quenched standards (Beckman[8]) and a TCB standard curve prepared for each tissue type.

Statistical analysis. All respiratory functions and [^{14}C]TCB uptake efficiency data were analyzed by a two-way analysis of variance (fish and time) with repeated measures on time (Winer, 1971). $p \leq 0.05$ was considered significant.

RESULTS

Respiratory Function

Specific measurements indicative of the respiratory function of both rainbow and brook trout are presented in Table 1. No sublethal effects at 0.10 μg/liter [^{14}C]TCB were observed on any of the respiratory functions measured, and close agreement was noted between individuals of each species. A significant species difference occurred only for ventilation rate ($p = 0.05$) with a 23% higher value recorded for the brook trout. The increased pumping rate of the brook trout did not, however, provide them with a larger ventilation volume than the rainbow trout when expressed on a per kilogram basis (Table 1).

Calculated Dose

The respiratory function measurements (Table 1) allowed calculation of the dose of TCB acquired by each fish exposed to a known water concentration of TCB over a set period of time (Table 2). These calculations were made by multiplying exposure time by ventilation volume (liters per hour) by the concentration of [^{14}C]TCB in water and by the [^{14}C]TCB uptake efficiency (%), which gave the total calculated micrograms of [^{14}C]TCB taken up in 48 hr. This value was converted to dose by dividing the total microgram value by individual fish weight.

No significant differences in the [^{14}C]TCB uptake efficiencies or in the ventilation volume were observed between species ($p = 0.05$) (Table 2). The dose of TCB/kg/48 hr calculated in Table 2 was the same in both species of trout.

The [^{14}C]TCB uptake efficiencies (%) (Table 2) were significantly higher ($p = 0.05$) than oxygen utilization (%) measurements (Table 1) indicating that [^{14}C]TCB was more readily taken up from the water than oxygen. It should also be mentioned that [^{14}C]TCB uptake efficiency (%) did not change with time over the 48-hr exposure period in any of the fish, which indicated a constant rate of TCB flux across the gills within the time period studied.

Measured Dose

To evaluate the accuracy of the calculated dose of [^{14}C]TCB from the water, the total ^{14}C accumulated by each fish was measured following 48-hr exposure to TCB (Table 3). This total body burden measurement was then added to the total ^{14}C excreted in the urine and in the feces to provide a mass-balance determination for [^{14}C]TCB absorbed across the gills of brook and rainbow trout exposed to a sublethal (0.10 μg TCB/liter) water concentration (Table 3). Expired water was also monitored for ^{14}C excreted across the gills, but none was detected. The calculated [^{14}C]TCB dose in Table 2 and the measured [^{14}C]TCB dose in Table 3 for all fish were extremely close with the maximum difference no greater than 10%.

Total Radioactivity in Body Fluids

The relative concentration of ^{14}C in both rainbow and brook trout body fluids was bile > whole body > plasma > red blood cells > urine. There were slightly higher concentrations of ^{14}C in rainbow trout for whole blood, plasma, red blood cells, and urine. There was significantly more ^{14}C in the plasma of both species than in the cellular fraction ($p = 0.05$).

Tissue Distribution of Calculated Dose

As indicated in Table 4, 96 to 103% of the [^{14}C]TCB dose in both brook and rainbow

TABLE 1

RESPIRATORY FUNCTION OF RAINBOW AND BROOK TROUT EXPOSED TO 0.10 μg [^{14}C]TCB/liter OVER A 48-hr PERIOD

Fish number	Sex	Weight[a] (kg)	Heart rate (beats/min)	Ventilation rate (No./min)	Ventilation volume (ml/kg/min)	Stroke volume[b] (ml)	Oxygen utilization[c] (%)	Oxygen consumption[d] (mg/kg/hr)
				Rainbow trout				
Control[e]		0.771 ± 0.07 (4)	41 ± 5 (2)	65 ± 4 (4)	144 ± 13 (4)	2.24 ± 0.27	58 ± 6 (4)	52.7 ± 5.3 (4)
1	F	0.669	—[f]	71 ± 4 (8)	200 ± 42 (8)	2.83 ± 0.48	47 ± 7 (8)	58.1 ± 4.5 (8)
2	F	0.839	—	63 ± 2 (8)	150 ± 17 (8)	2.40 ± 0.26	60 ± 2 (8)	58.0 ± 5.2 (8)
3	F	0.767	—	66 ± 2 (8)	142 ± 10 (8)	2.15 ± 0.12	54 ± 2 (8)	48.7 ± 2.2 (8)
4	M	0.793	35 ± 4 (3)	61 ± 3 (8)	121 ± 9 (8)	1.98 ± 0.15	64 ± 2 (8)	49.5 ± 3.5 (8)
Overall mean		0.771[g] ± 0.07 (4)		65 ± 4 (4)[h]	151 ± 22 (4)	2.31 ± 0.26	56 ± 7 (4)	53.6 ± 5.2 (4)
				Brook trout				
Control[e]		0.613 ± 0.06 (5)	48 ± 9 (3)	85 ± 12 (5)	131 ± 28 (5)	1.53 ± 0.26	66 ± 9 (5)	54.4 ± 7.9 (5)
5	M	0.692	38 ± 2 (8)	63 ± 3 (8)	146 ± 10 (8)	2.32 ± 0.16	56 ± 2 (8)	53.4 ± 4.0 (8)
6	M	0.532	—	90 ± 2 (8)	154 ± 19 (8)	1.71 ± 0.24	60 ± 4 (8)	59.5 ± 6.6 (8)
7	F	0.621	52 ± 8 (8)	77 ± 4 (8)	122 ± 15 (8)	1.58 ± 0.27	81 ± 4 (8)	64.1 ± 5.1 (8)
8	F	0.559	64 ± 3 (7)	91 ± 4 (9)	188 ± 29 (8)	2.07 ± 0.36	51 ± 4 (9)	60.5 ± 5.3 (8)
9	F	0.642	—	98 ± 4 (9)	150 ± 9 (9)	1.53 ± 0.11	67 ± 3 (9)	63.4 ± 5.8 (8)
Overall mean		0.613 ± 0.06 (5)	51 ± 13 (3)	84 ± 14 (5)[h]	150 ± 21 (5)	1.79 ± 0.47	63 ± 12 (5)	60.2 ± 4.3 (5)

[a] Measured before exposure.
[b] Ventilation volume ÷ by ventilation rate.
[c] Inspired (DO) minus expired (DO) ÷ inspired (DO) × 100.
[d] Mean ventilation volume × mean incoming DO × mean oxygen utilization ÷ fish weight (kg).
[e] Mean of all fish before exposure to TCB, based on at least three measurements per fish.
[f] No data.
[g] Mean, standard deviation, and total *N* (in parentheses).
[h] Overall means for brook and rainbow trout significantly different ($p = 0.05$).

TABLE 2

CALCULATION OF A [^{14}C]TCB DOSE ACQUIRED BY RAINBOW AND BROOK TROUT EXPOSED TO WATERBORNE [^{14}C]TCB OVER A 48-hr PERIOD

Fish number	Exposure time (hr)	×	Ventilation volume (liters/hr)	×	[^{14}C]TCB in water (μg/liter)	×	[^{14}C]TCB uptake efficiency[b] (%)	=	Total calculated [^{14}C]TCB[c] (μg/48 hr)	÷	Fish weight (kg)	=	Calculated [^{14}C]TCB dose[d] (μg/kg/48 hr)
							Rainbow trout						
1	48		8.06[a] ± 1.71		0.084 ± 0.004		82 ± 3		26.65		0.669		39.83
2	48		7.58 ± 0.85		0.084 ± 0.004		84 ± 3		25.67		0.839		30.60
3	48		6.56 ± 0.47		0.101 ± 0.004		70 ± 3		22.25		0.767		29.02
4	48		5.76 ± 0.40		0.099 ± 0.002		86 ± 5		23.54		0.793		29.68
									$\bar{x}$ SD 24.5 ± 2.0				$\bar{x}$ SD 32.3 ± 5.0
							Brook trout						
5	48		6.06 ± 0.41		0.069 ± 0.003		77 ± 6		15.45		0.692		22.33[e]
6	48		4.89 ± 0.62		0.107 ± 0.005		68 ± 7		17.08		0.532		32.10
7	48		4.55 ± 0.54		0.102 ± 0.004		79 ± 8		17.60		0.621		28.34
8	48		6.31 ± 0.95		0.099 ± 0.006		60 ± 5		17.99		0.559		32.18
9	48		5.75 ± 0.35		0.097 ± 0.006		79 ± 4		21.15		0.642		32.94
									$\bar{x}$ SD 17.8 ± 2.1				$\bar{x}$ SD 31.4 ± 2.1

[a] $\bar{x}$ ± SD of eight or nine measurements.
[b] Uptake efficiency = ([^{14}C]TCB inspired − [^{14}C]TCB expired)/([^{14}C]TCB inspired) × 100.
[c] Exposure time × ventilation volume × water concentration × uptake efficiency.
[d] Total calculated TCB ÷ fish weight.
[e] Not included in calculation of mean because of low TCB water concentration.

TABLE 3

DETERMINATION OF THE ACTUAL MEASURED [^{14}C]TCB DOSE RECEIVED BY BROOK AND RAINBOW TROUT DURING A CONTINUOUS 48-hr EXPOSURE TO WATERBORNE [^{14}C]TCB

Fish number	[^{14}C]TCB[a] in carcass[b] (μg)	+	Total [^{14}C]TCB[a] urine (μg)	+	Total [^{14}C]TCB[a] feces (μg)	=	Total measured[c] [^{14}C]TCB (μg)	÷	Fish weight (kg)	=	Measured[d] [^{14}C]TCB dose (μg/kg/48 hr)
					Rainbow trout						
1	25.76		0.090		0.058		25.9		0.669		38.72
2	26.76		0.256		0.029		27.0		0.839		32.23
3	24.49		0.238		0.058		24.7		0.767		32.31
4	24.37		0.252		0.092		24.7		0.793		31.16
							$\bar{x}$ SD 25.6 ± 1.1				$\bar{x}$ SD 33.6 ± 3.5
					Brook trout						
5	15.01		0.117		0.054		15.18		0.692		21.94[e]
6	15.92		0.161		0.023		16.10		0.532		30.27
7	17.95		—		0.133		18.08		0.621		29.12
8	17.87		0.107		0.117		18.09		0.559		32.36
9	18.20		0.096		0.086		19.18		0.642		29.87
							$\bar{x}$ SD 17.4 ± 1.6				$\bar{x}$ SD 30.4 ± 1.4

[a] Total [^{14}C]TCB is the parent compound plus metabolites.
[b] Carcass includes whole fish plus [^{14}C]TCB in bile and blood samples taken.
[c] Sum of μg [^{14}C]TCB in carcass, urine, and feces.
[d] Total measured [^{14}C]TCB ÷ fish weight.
[e] Not included in calculation of mean because of low TCB water concentration.

TABLE 4

PERCENTAGE OF THE CALCULATED DOSE OF [^{14}C]TCB MEASURED IN THE CARCASS, WHOLE BLOOD, BILE, URINE, AND FECES OF BROOK AND RAINBOW TROUT EXPOSED FOR 48 hr TO 0.100 μg TCB/liter

		Percentage of total calculated dose[a]						
				Urine				
Fish number	Carcass	Whole blood	Bile 48 hr	24 hr	48 hr	24 + 48 hr total	Feces 48 hr	Total[b]
				Rainbow trout				
1	96.73	0.17	1.42	0.08	0.26	0.34	0.22	98.88
2	104.24	0.20	1.14	0.48	0.52	1.00	0.10	106.68
3	109.98	0.10	1.36	0.47	0.60	1.07	0.20	112.71
4	103.51	0.10	0.99	0.40	0.68	1.08	0.39	106.07
Overall mean	103.62 ± 5.43	0.14 ± 0.05	1.23 ± 0.20	0.36 ± 0.19	0.52 ± 0.18	0.87 ± 0.36	0.23 ± 0.12	106.09 ± 5.66
				Brook trout				
5	97.12	0.09	1.94	0.46	0.30	0.76	0.35	100.26
6	93.22	0.18	0.87	0.43	0.52	0.95	0.14	95.36
7	101.98	0.23	0.63	—[c]	—[c]	—[c]	0.76	103.60
8	99.30	0.20	1.14	0.19	0.41	0.60	0.65	101.92
9	89.82	0.13	1.17	0.25	0.20	0.45	0.41	91.98
Overall mean	96.29 ± 4.83	0.17 ± 0.06	1.15 ± 0.49	0.33 ± 0.13	0.36 ± 0.14	0.69 ± 0.21	0.46 ± 0.25	98.62 ± 4.82

[a] See Table 2.
[b] Sum of all category percentages for each fish.
[c] Not measured.

trout acquired over 48 hr resided in the carcass, which included all major organ systems, muscle, skin, and fat. Excretory losses (urinary and fecal) of [^{14}C]TCB from rainbows and brook trout over the 48-hr exposure period amounted to 1.1 and 1.2%, respectively. Bile remaining in the gall bladders of both species contained 1.2% of the total [^{14}C]TCB.

To see how closely calculated and measured determinations agreed, the total micrograms of ^{14}C in each tissue (Table 3) was expressed as a percentage of the calculated dose (Table 2) and then the percentages were totalled in the last column of Table 4. These percentages varied less than 10%.

DISCUSSION

The use of the pure isomer 2,5,2′,5′-[^{14}C]tetrachlorobiphenyl, a lipophilic, persistent, slowly metabolized chemical, provided us with an excellent chemical for conducting controlled mass-balance studies with fish. Thus, we were able to evaluate the accuracy and validity of dose determinations and to measure the pharmacokinetic aspects of TCB in two species of trout for use in aquatic toxicology.

The mean calculated dose of [^{14}C]TCB obtained from a water concentration of 0.084 to 0.107 μg TCB/liter was 32.3 μg/kg/48 hr for rainbow trout and 31.4 μg/kg/48 hr for brook trout, while the mean measured dose was 33.6 μg/kg/48 hr for rainbow and 30.4 μg/kg/48 hr for brook trout. This close agreement between calculated and measured doses showed that direct measurements of chemical flux across the gills can be accurately and reproducibly made for waterborne chemicals. These results also further verified our earlier work on the direct gill uptake of waterborne endrin by brook trout, involving dose determinations made at two different water concentrations of endrin (McKim and Goeden, 1982).

The total flux of a chemical across the gills is a function of water concentration, uptake efficiency, and respiratory volume. Since the uptake efficiency remains constant as described for endrin by McKim and Goeden (1982), the amount taken up depends on the concentration in the water and the total volume of water pumped over the gills. This relationship was shown in the present study by the data for fish number five, where the calculated and measured doses were identical yet below the values for the other fish, because of the lower water exposure concentration of TCB.

The mean uptake efficiency of [^{14}C]TCB (76%) for both species was greater than oxygen utilization (60%), indicating that 76% of the TCB in the inspired water was removed on one pass across the gills, while only 60% of the oxygen present was removed. This greater affinity for TCB than for oxygen was identical to the relationship seen for endrin (80%) and oxygen (60%) (McKim and Goeden, 1982). Weininger (1978), however, considered the uptake of oxygen and polychlorinated biphenyls (PCB) across the gills to be equal based on the diffusion of oxygen and PCB. Why TCB and endrin in our studies should differ from Weininger's calculations is unknown, but it could be caused by uptake via the oral membranes of our fish in addition to the flux across the gills.

Respiratory function was consistent among individuals of each species, and no effects of TCB on respiratory function were observed. There were also no significant differences in normal respiratory function between the brook and rainbow trout except for a more rapid respiratory rate for brook trout. All respiratory function measurements made on transected brook trout were the same as those reported earlier by McKim and Goeden (1982). In addition, the literature values reported for untransected brook and rainbow trout respiratory function were similar to those reported here.

The high mean gill uptake efficiency of [^{14}C]TCB (76%) for both species was coupled

with a very low excretion rate (1%). No ^{14}C was excreted from the gills of either species. This lack of excretion was also confirmed by the close agreement of measured and calculated doses. Therefore, of the total ^{14}C excreted by both species, 75% was in the urine, while 25% was in the feces. The major excretory routes of TCB for both rainbow and brook trout were the bile and urine. Although they did not sample urine, Guiney *et al.* (1977) also suggested the bile was a major elimination route in rainbow trout. Sanborn *et al.* (1975) pointed out that green sunfish rapidly metabolize and excrete 2,5,2′-trichlorobiphenyl, while TCB was only very slowly metabolized and excreted. Hutzinger *et al.* (1972) found no metabolism of TCB in brook trout, while Melancon and Lech (1976) found very slow metabolism in rainbow trout. Further metabolism–excretion studies on rainbow trout by Guiney *et al.* (1977) showed a rapid 20% loss from a "mobile" (rapidly excreted) compartment, involving primarily the blood and bile, followed by a very slow loss from a deeper compartment similar to the loss seen in the present study. We saw no large mobile compartment, possibly because a much lower continuous TCB exposure concentration was used. Guiney's relatively large single dose resulted in a much larger initial "mobile" phase than our fish experienced. Other researchers (Hansen *et al.*, 1971; Hattula and Karlog, 1973) observed a rapid excretion of PCBs (60 to 80%) from previously exposed fish. However, their fish were exposed to complex mixtures of polychlorinated biphenyls, and Gage and Holm (1976) showed that certain PCB isomers were more rapidly metabolized and excreted because of the position and lower number of chlorine atoms on the molecule.

We observed that approximately 98% of the 48-hr dose resided in the major organs and muscle tissues of these fish as described earlier for salmonids by Guiney *et al.* (1977) and Lieb *et al.* (1974), while approximately 2% was present in blood, bile, urine, and feces. The proportion in the blood was more heavily concentrated in the plasma than in the red cells, as was also reported for hexachlorobiphenyl in rats by Muhlebach and Bickel (1981).

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